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Metachaos granulosa in Kansas; Third Recorded Appearance of Gruber's Rare Ameba.

M. ANTHONY PAYNE,
Department of Zoölogy, University of Kansas.

I.

THIS ameba, whose first appearance was recorded in Germany by Gruber in "Studien über Amöben," 1885, was again observed and described in France by Penard, 1902. It has been found for the third time, in Kansas, in fresh laboratory cultures which were set up at the State University in the early part of June.

1. Gruber (*2*) described many species of amebas. He treated, in great detail, "*Amœba proteus*" (*Polychaos dubia?*) and several other species which he called "prima," "secunda" "tertia," etc. Then he mentioned a collection of smaller amebas with which he did not greatly concern himself, but which were of interest. In this collection there were two kinds of amebas which he watched as long as he could keep them alive, and then he recorded the fact that they were very different types. One he called (*Amœba*) *spumosa;* the other (*Amœba*) *granulosa.*

He writes that *granulosa* is about 0.03 mm. in thickness. The body is filled with a great number of clear refractive elliptic granules through which a vesicular nucleus may be seen. There is a single contractile vacuole and some food bodies.

In 1885 Gruber complained because too many species of amebas had been inadequately described. He said that it was hard to classify amebas because no secure basis for classification had been arranged. Nevertheless, he recognized that there were different kinds of amebas, and perhaps he realized that these maintained their individuality of structure, for he speaks of "Körper Merkmale" and of characteristic granules. At any rate, he did not hesitate to

name a *number* of *new species,* among them (*A.*) *granulosa,* when markedly different organisms appeared in his cultures. Plate XXVIII, figure 1, is a reproduction of his drawing of *granulosa.*

2. Seventeen years later, Penard described (*A.*) *granulosa* Gruber in much greater detail. His scientific observations and illustrations (Pl. XXVIII, figs. 2-4) are a great addition to the meager remarks of Gruber. Penard, following the lead of Gruber, characterizes the species by means of the nucleus and the immense number of small crystals which fill the body—"d'une quantite immense de petits grains reguliers elliptiques, qui remplissent le corps presque completement et paraissent de nature siliceuse" (Pl. XXVIII, fig. 2). He says that the crystals are always regular and pointed at both ends. At first they seem to be simple ellipses, but on close examination it is evident that they are bicuspid and that the edges are rounded off. He notes the high refractive index, as well as the color: "They are a brilliant yellow, but give the body of the ameba a cast of dark violet when they are examined under high power.

Some time later Penard found more amebas in a culture which he obtained from a marsh mear Gaillard. These measured from 300 μ to 500 μ and were at first grouped with "*A. proteus*" (*P. dubia*). Later he decided that they approached nearer to (*A.*) *granulosa,* because they were filled with "de petits grains bicuspides."

Mention is made of a contractile vacuole which is always seen at the posterior end of the organism. The vesicular spherical nucleus is generally about 50 μ (Pl. XXVIII, fig. 14). It has a clear and strong membrane and the chromatin material, consisting of small grains, is arranged in a layer immediately under the membrane. The illustration of the nucleus (Pl. XXVIII, fig. 4) is doubtlessly a polar view, not an optical section.

The movement, generally very rapid, is compared to that of "*A. proteus.*" Large pseudopods are produced on various parts of the body, and the endoplasm, with its inclusions, quickly penetrates these. The length of the organism, when still, is 300 μ. When flowing it may measure 500 μ. Extremely fine, dustlike granules are mentioned among the inclusions of the protoplasm; also brilliant bodies, yellow or greenish, globular in form or variable. Penard says that these may play the part of spore bodies.

3. Cash (*1*) cites a *granulosa* as a variety of "*A. proteus,*" but it is evident from his figures that he is confusing *Chaos diffluens* with (*A.*) *granulosa* Gruber. The shape of the nucleus as well as the pronounced mulberry-shaped caudal extremity, as Cash has them,

are not characteristic of *granulosa*. Cash refers to Leidy's figure (*3*) in confirmation of his assertion, but from Schaeffer's ('20) research and differentiation (*5*) it is evident that both Cash and Leidy were dealing with *Chaos diffluens*, and probably also with *Metachaos discoides*.

II.

1. There is some question as regards the exact source of the local culture in which *granulosa* appeared at the University of Kansas. They were first observed in two watch glasses of green scum which came from the entomology greenhouse. Here a slow stream of water flows through a series of cement water basins in which blue-green algæ are growing. Scum and debris were collected and brought to the laboratory and distributed in culture dishes and watch glasses, which were half filled with hydrant water. In about three weeks there was a score of unfamiliar amebas in two of the watch glasses. After they were studied for some time it was concluded that they were the species which Penard called (*A.*) *granulosa* Gruber.

2. (*a*) This *Metachaos granulosa* most closely resembles *M. discoides*. It is from 200 μ to 250 μ in length and is densely filled with refractive granules, which often give the body a pronounced cerulean tint. It is fast-moving, a rate of 250 μ per minute having often been observed. (Pl. XXVIII, figs. 7, 8.) Very few lateral pseudopods are formed during locomotion. Wave after wave of clear hyaline ectoplasm is thrown out at the anterior end. The granular endoplasm follows. The manner of streaming is similar to that observed in the large amebas, *Chaos diffluens* and *Metachaos discoides*, especially the latter. The hyaline margin of ectoplasm is wider at the anterior end than that observed in closely related species. The hyaline margin or band becomes narrower toward the posterior end of the amebas, where it is hardly perceptible. The ectoplasmic surface is smooth, as in *discoides*. The large, well-defined contractile vacuole, pulsating about every 3.5 minutes (temp. 26° C.) is always at the extreme posterior part of the cell. A uroid is not present and the ameba never carries debris. A very common body form is limned by means of a posterior and lateral pseudopod which is retained for long periods (Pl. XXVIII, figs. 5, 6).

(*b*) The crystals, mentioned by Gruber and described by Penard, are a *nominal* outstanding characteristic. As mentioned before, they are so closely packed that when the light is diminished and the amebas are observed under low power the crystals cause the amebas to appear of a cerulean tint. The shape of most of the crystals is

similar to those found in *discoides*. A polar view shows plainly the square base of its pyramidal form, while a side view shows the bipyramidal form. Together with these are found the bipyramidal crystals "pointed on both ends" (Pl. XXVIII, figs. 9, 10). An estimate of the percentage of each was not obtained, but there seemed to be more of the truncated variety.

Intermingled with the crystals are numerous—perhaps 200—so-called excretion spheres. Many are over 1 μ in diameter. Large food vacuoles are not present. No definitely recognized food masses were seen in any of the specimens.

(*c*) In the last analysis, it is the nucleus which most sharply separates *granulosa* from *discoides* and other species. To quote Schaeffer ('20), "No two species are alike in any respect whatsoever" (*5*). It does not require the expert's trained eye to see the difference in the nuclei of *discoides* and *granulosa* (see Pl. XXVIII, figs. 12, 14). *Metachaos granulosa* has a spherical nucleus; it is never a biconcave disc. The diameter of the nucleus is about 25 μ. A clear membrane surrounds the chromatin material, which appears as a single layer of small spheres at a uniform depth. A polar view of the nucleus and an optical section are shown in (Pl. XVIII, fig. 14.)

III.

These descriptions are based on somewhat meager data, for the culture was not successfully established; hence the records cannot be enlarged upon or accurately checked. Extensive research has been made on cultures similar to the ones first prepared, but *granulosa* amebas were not again discovered. It is just barely possible that the amebas were introduced from other local cultures, for the glassware was not sterilized. It has, nevertheless, been thought worth while to record the appearance of this interesting ameba, for when once it is seen and examined the distinctive characteristics are such that it is recognized as *granulosa*. It is probable, too, that there are more of these amebas in the Kansas distribution and these data may call attention to them.

LITERATURE.

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EXPLANATION OF PLATE XXVIII.

FIG. 1.—Gruber's *Amœba granulosa.*

FIGS. 2 TO 4.—Penard's illustrations, *"Amœba granulosa."*

2. Animal in motion.
3. Details of the protoplasm.
4. Nucleus.

FIGS. 5 TO 8.—Laboratory specimens.

5. An odd specimen, the anterior half filled with excretion spheres. the posterior part crowded with crystals.
6. Normal specimen.
7, 8. Paths of locomotion.

FIGS. 9 AND 10. Crystals contained in the cytoplasm.

9. Truncated bipyramidal crystals, showing polar views and side views.
10. Bipyramidal crystals.

FIGS. 11 TO 14. Comparative table of nuclei.

11. *Chaos diffluens:* A, Polar view of the nucleus. B, Equatorial view of the discoid nucleus. C, Equatorial view of a folded or crushed nucleus.
12. *Metachaos discoides:* A, Equatorial view of nucleus. B, Polar view.
13. *Polychaos dubia:* A, Equatorial view of ovoid nucleus. B, Polar vief of same.
14. *Metachaos granulosa:* A, Polar view. B, Optical section.

Dimensions in microns: 11-B, 46 x 12; 12-A, 40 x 18; 13-A, 40 x 32; 14-A, 25 x 25.

(Figures 11 to 13 after Schaeffer.)
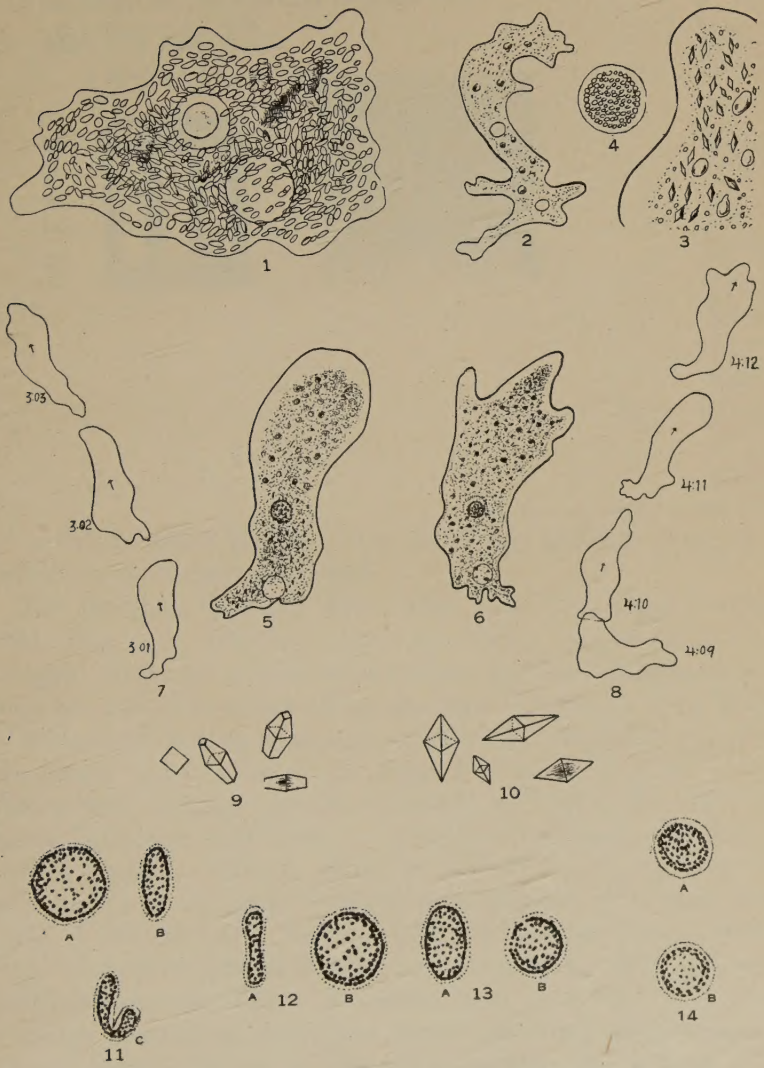
PLATE XXVIII.